J Chem Ecol (2007) 33:2308–2324
DOI 10.1007/s10886-007-9394-y

Scented Males and Choosy Females: Does Male Odor Influence Female Mate Choice in the Mediterranean Fruit Fly?

Todd E. Shelly · James Edu · Elaine Pahio · Jon Nishimoto

Received: 8 June 2007 / Revised: 9 October 2007 / Accepted: 17 October 2007 /
Published online: 21 November 2007
© Springer Science + Business Media, LLC 2007

Abstract The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), displays a lek mating system characterized by a high level of female discrimination among potential mates. The basis of female choice is not understood, but recent studies indicate that male exposure to the aroma of certain plant structures or essential oils may increase mating success. In particular, exposure to the aroma of ginger root oil (GRO) enhances male mating frequency, and several sterile-male release programs against *C. capitata* have incorporated 'aromatherapy' (large-scale exposure of pre-release insects to GRO) to increase the effectiveness of control efforts. We investigated the mechanism underlying female preference for GRO-exposed males. Two sets of experiments were conducted. In the first, we monitored female attraction to (1) freshly killed flies, or (2) paper discs that contained hexane extracts from varying treatments. In these tests, females were sighted more often (1) near GRO-exposed than non-exposed males (even when the males were visually concealed) and (2) near extracts from GRO-exposed than non-exposed males. These findings suggest a 'perfume effect', whereby female mate choice is mediated by olfactory differences. In the second set, we compared (1) mate choice between intact females and females from which both antennae had been surgically removed, and (2) mating success between intact males and males from which both antennae had been surgically removed before GRO exposure. Intact females preferred GRO-exposed males, whereas females lacking both antennae rarely mated and showed no preference between GRO-exposed and non-exposed males. In the opposite treatment (intact females but surgically altered males), GRO-exposed males lacking both antennae mated as frequently as GRO-exposed intact males. These data suggest that female choice was dependent on olfactory perception of male odor but that male mating success did not depend on olfactory perception of GRO aroma, suggesting, in turn, that GRO conferred a mating advantage through an external phenomenon (possibly alteration of cuticular scent) rather than through internal processing (pheromone synthesis).

T. E. Shelly (✉) · J. Edu · E. Pahio · J. Nishimoto
USDA-APHIS, 41-650 Ahiki Street, Waimanalo, HI 96795, USA
e-mail: todd.e.shelly@aphis.usda.gov

Keywords *Ceratitis capitata* · Mediterranean fruit fly · Tephritidae · Mate choice · Ginger root oil · Pheromones

Introduction

Phytochemicals influence the mating behavior of phytophagous insects in two main ways. Specific compounds that occur in host plants may signal the presence of food or oviposition resources to females; males, responding to the same signals, high female traffic, or both, may aggregate at the same sites because they offer high probability of encountering potential mates (Thornhill and Alcock 1983; Bernays and Chapman 1994). Males of many phytophagous insects aggregate at the primary feeding and oviposition sites of females, and chemical stimuli may guide males, not only to the appropriate plant species (Quiroz et al. 2005) but also to plants or structures of a given host species that is preferred by females. For example, males and females of an oligophagous, desert-dwelling grasshopper tend to settle preferentially on those creosote bushes (the primary food resource) whose leaves contain relatively low amounts of the potent antioxidant nordihydroguaiaretic acid (Greenfield et al. 1987). Males of a longhorn beetle species respond to the odor of eucalyptus logs, where females aggregate for egg-laying (Hanks et al. 1996; see also Ginzel and Hanks 2005). Male attraction to host plant volatiles, leading to increased encounter rates with receptive, pre-ovipositing females, has also been reported in moths (Coracini et al. 2004) and bark beetles (Hovorka et al. 2005).

In addition to affecting male and female spatial distributions, plant chemicals may have a more subtle effect on insect mating systems through mediation of the production and release of sex pheromones (Landolt and Phillips 1997). For example, particular plant chemicals ingested by larvae (Connor et al. 1981; Lofstedt et al. 1989) or adults (Pliske 1975; Krasnoff and Dussourd 1989) may serve as precursors for pheromone synthesis. Feeding on the host plant may also stimulate production of male sex pheromone (Hughes and Renwick 1977), and exposure to host plant volatiles may trigger pheromone release (Jaffe et al. 1993). In addition, plant odors may affect female responsiveness to male sex pheromones. In various taxa, including flies (Bartelt et al. 1988), beetles (Dowd and Bartelt 1991), and moths (Landolt et al. 1994), female attraction to male pheromone is enhanced in the presence of host plant odors.

These main effects of plant chemicals—influencing mate location and intersexual communication—appear to be important in shaping the lek mating system (Prokopy and Hendrichs 1979) of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Tephritidae). Males aggregate in the canopy of host and nonhost trees and defend individual leaves as mating territories. Whereas perching on the leaf undersurfaces, males engage in 'calling behavior' in which the abdomen is curled upward, a bubble-like structure (the rectal epithelial sac) is everted, and a sex pheromone is emitted (Arita and Kaneshiro 1986). The pheromone is a complex blend that contains three to five major components known to be attractive to females (Heath et al. 1991; Jang et al. 1994) plus at least 50 minor components whose function has not yet been elucidated (Jang et al. 1989). After female arrival at a male perch, the male initiates vigorous wing movements coupled with side-to-side head rocking (Féron 1962). If the female remains stationary, the male mounts and attempts to copulate. Copulation generally lasts 2–3 hr (Whittier et al. 1992). Females are highly selective and reject courting males in more than 90% of laboratory observations by moving away during male signaling or dropping from the substrate after male mounting (Whittier et al. 1994).

Regarding intersexual encounter sites, field data (Hendrichs and Hendrichs 1990) showed that mating aggregations (leks) were non-randomly distributed among available host trees, with most matings (49%) occurring on orange trees (*Citrus sinensis* L.) although this host represented only a small fraction (12%) of all available host trees in the habitat. Furthermore, within the canopy of individual trees, both sexes settled in greater numbers in the vicinity of oranges than in locations having comparable physical characteristics (e.g., light level, temperature, and leaf density) but no fruits (Shelly and Kennelly 2007). Although visual stimuli are probably involved as well, strong attraction to citrus volatiles has been demonstrated for both sexes of *C. capitata* under field conditions (Katsoyannos et al. 1997).

Recent work has shown that plant-derived chemicals may also influence the mating success of male medflies. Papadopoulos et al. (2001) and Shelly et al. (2004a) found that *C. capitata* males exposed to oranges obtained significantly more copulations than non-exposed males. In one case, the exposed males obtained 66% of the total matings (Shelly et al. 2004a). Similarly, male medflies exposed to the bark and fruits of guava trees (*Psidium guajava* L.) had a mating advantage over males deprived access to these substrates (Shelly and Villalobos 2004). Using commercially available oils, Shelly et al. (2004a, b) and Papadopoulos et al. (2006) further demonstrated that the aroma of ginger root oil (*Zingiber officinale* Roscoe) and orange oil conferred a mating advantage to male medflies. Encouraged by these latter results, Shelly et al. (2007a) have promoted the implementation of 'aromatherapy' (large-scale exposure of flies to ginger root oil) to increase the mating competitiveness of medfly males used in sterile release programs.

It is not known why exposure to certain fruits or oils enhances the mating competitiveness of male medflies. Most of the work conducted thus far has involved male exposure to the aroma of ginger root oil (GRO hereafter), and several conclusions can be drawn. Males exposed to GRO spend a greater proportion of their time pheromone-calling than non-exposed males (Shelly 2001; Papadopoulos et al. 2006). However, the relative increase in calling activity appears insufficient to explain the magnitude of the observed mating increase. For example, Shelly (2001) found that GRO-exposed males obtained approximately twice as many matings as non-exposed males, but that the level of pheromone-calling displayed by GRO-exposed males was only about 30% higher than that observed for non-exposed males. Subsequent trials with a wind tunnel confirmed that females were equally attracted to the pheromone of GRO-exposed and non-exposed males (Papadopoulos et al. 2006). Thus, whereas GRO exposure results in a slight increase in male signaling activity, it does not appear to affect the attractiveness of the pheromone signal to females.

The present study further investigates the mechanism that underlies the increased mating success of GRO-exposed males of *C. capitata*. In particular, we present the results of two sets of behavioral experiments that examined the possibility that female preference for GRO-exposed males was mediated by a 'perfume effect', whereby the aroma of GRO affected the male cuticle in some manner that enabled females to distinguish between exposed and non-exposed individuals. In the first set of experiments, we presented females with freshly killed flies or hexane extracts from flies and monitored female presence near the different treatments. By offering such stimuli, we eliminated the possibility that active displays, behavioral or pheromonal, influenced female response. In the second set, we compared mate choice between intact females and females from which one or both antennae were surgically removed. By removing the primary olfactory receptors, we directly examined the role of male odor in affecting female choice. We also conducted the converse experiment, i.e., we compared mating success between non-

exposed males and GRO-exposed males that were intact or in which one or both antennae were surgically removed before the exposure. By performing this surgery, we directly examined the importance of male perception of the GRO odor in influencing mating success.

Methods and Materials

Study Insects Medflies used in this study were derived from a laboratory colony started with >500 adults reared from coffee (*Coffea arabica* L.) berries collected on the island of Kauai, Hawaii. The colony was four to five generations removed from the wild when this study was conducted. Adults of *C. capitata* were held in screen cages and provided a mixture of sucrose and yeast hydrolysate (3:1, *v*/*v*) in a Petri dish, water, and oviposition substrate (perforated plastic vials containing small sponges soaked in lemon juice). Eggs were placed on standard larval medium (Tanaka et al. 1969), and mature larvae 'popped' onto a layer of vermiculite for pupation. Adult medflies used in the experiments were separated by sex within 48 hr of eclosion, well before reaching sexual maturity at 6–8 d of age. When used in the experiments, males were 9–13 d old and females were 10–15 d old. Flies were held in screen-covered, plastic containers (5 l; 125–150 flies per container) at 23–26°C and 60–90% RH and received both natural and fluorescent light on a 12 L/12 D photoperiod. Experimental flies were maintained on the same sugar-yeast hydrolysate mixture used for the colony.

In several experiments, we also used males of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), derived from a laboratory colony started with >200 adults reared from guava (*Psidium guajava* L.) fruits collected on the island of Oahu, Hawaii. The colony was two generations removed from the wild when this study was conducted. The oriental fruit fly colony was maintained in much the same manner as the medfly colony, except that eggs were deposited and larvae developed in fruit (papayas, *Carica papaya* L.). When used in the experiments, *B. dorsalis* males were 22–30 d old (sexual maturation in this species occurs between 15–21 d of age).

Female Response to Dead Flies: Basic Protocol Experiments in which we monitored the response of *C. capitata* females to various treatments of freshly killed flies followed the same basic protocol (with the single exception of experiment 11, see below). On a given test day, 30 females were transferred (with an aspirator) from the holding containers to screen cages (30 cm cubes) between 0830–0900 hours and held (in a different room from the test room) until tested between 1000–1130 hours. Groups of ten 'stimulus' flies from the treatments being compared were transferred from holding containers to a freezer for 15–20 min; flies from the different treatments were placed in separate freezers. Upon removal from the freezer, dead flies were held at room temperature for 10 min, transferred to plastic Petri dishes (5.4 cm diameter) and introduced into the test cages containing the females. Thus, for all trials, a given screen cage contained 30 females and two Petri dishes (10 cm apart), each holding ten freshly killed flies from the two treatments being compared.

Starting 1 min after placement of the stimulus flies, we recorded the number of females resting on each of the dishes at 1-min intervals over a 30-min period. Because females were not marked, the number of sightings recorded represented a composite index that encompassed both female arrival to and retention on a particular dish. An observer

monitored two cages simultaneously (containing the same two treatments of stimulus flies) and one pair of cages per day (with different, randomly selected treatments of stimulus flies in the first and second pairs). During observations, the cages were resting on a table (0.5 m apart) adjacent to a window. For a given experiment, the locations (right or left from the observer's vantage) of the two treatments within the test cages were alternated among the replicates to control for a possible position effect. Females and stimulus flies were used for a single trial only, and the test cages and Petri dishes were rinsed with water at the end of daily observations.

For several experiments, we compared a given pair of treatments first by using uncovered dishes, and then we conducted a second set of replicates by using covered dishes (i.e., a 5 cm square of white cotton cloth over the stimulus flies). By preventing females from seeing or touching the stimulus flies, the cloth cover was used to isolate more completely the role of olfactory cues in influencing female settlement.

Female Response to Dead Flies: Species- and Sex-Specific Attraction We first investigated species- and sex-specific attraction of *C. capitata* females. In experiment 1, females were offered conspecific males and *B. dorsalis* males, and in experiment 2, females were offered conspecific males and females.

Because females showed strongest attraction to conspecific males, we conducted three additional experiments to determine whether conspecific males (not exposed to GRO) had hexane-extractable compounds that were attractive to females. In experiment 3, we monitored female response to paper discs (8 mm diameter) treated with hexane extract from male medflies *vs* discs treated with hexane only. We placed ten chilled males in 0.25 ml of hexane for 1 min, removed them, and then placed a paper disc in the extract until the extract was entirely absorbed. The disc was then dried for 1 hr before testing. The 'control' discs absorbed the same volume of hexane and were dried for 1 hr before use. During the trials, discs were presented in Petri dishes following the standard procedure.

In experiment 4, we compared female sightings between hexane-washed and non-washed males (neither group was exposed to GRO). In this case, washed males were chilled, placed individually in glass beakers that contained 2 ml of hexane, shaken gently for 30 sec, and dried for 1 hr before testing. Because this treatment appeared to remove the volatile attractants, given that hexane-washed males were less attractive to females than non-washed males, we performed experiment 5 to examine the possibility that females were simply avoiding the hexane-washed males. We compared female attraction to two dishes of (non-exposed, uncovered) conspecific males, one of which also contained ten hexane-washed males (obtained following the protocol of the preceding experiment) beneath a cloth cover and the other had only a cloth with no flies beneath (the cloth covers used in this experiment were 2 cm squares, and thus covered only a portion of the dish).

Female Response to Dead Flies: GRO-Exposure to Conspecific Males We next investigated the influence of GRO exposure on the attraction of *C. capitata* females. In experiment 6, we offered females either GRO-exposed or non-exposed conspecific males. To obtain exposed males, we applied 0.25 ml of GRO to a cotton wick, placed the wick in a container (5 l) that held 30 males, and then removed it 2 hr later. Adjusted for container volume, this dose was equivalent to one previously demonstrated to increase male mating performance (Shelly 2001). GRO exposure was conducted in an isolated room between 1000–1200 hours; flies were exposed 1 d before testing (i.e., flies were freeze-killed and bioassayed the following morning).

Because females were more frequently observed near the GRO-exposed than the non-exposed males and given the results of experiment 4, we considered the hexane-wash procedure to be an effective means of extracting compounds responsible for attraction. Therefore, in experiment 7, we repeated the protocol of experiment 6 but (after chilling) extracted the GRO-exposed males with hexane following the procedure described above for experiment 4.

Additional Tests on the Impact of GRO Exposure on Female Response Four experiments were conducted to investigate additional aspects of how GRO exposure affects female attraction.

In experiment 8, we monitored the response of females to paper discs treated with hexane extract from GRO-exposed males *vs* non-exposed males. The GRO exposure and hexane washing procedures followed those described for experiments 6 and 3, respectively.

In experiment 9, we compared female response to conspecific males that had no direct GRO exposure but were confined with GRO-exposed males immediately before testing. This experiment investigated the possibility that the attractant could be transferred among crowded individuals, a phenomenon demonstrated in several *Drosophila* species (Coyne et al. 1994; Marcillac and Ferveur 2004). We placed ten GRO-exposed males (exposure followed the same procedure described for experiment 6, except that in this instance the males were chilled just before GRO exposure and the distal tip of the right wing was clipped for identification) and ten non-exposed males in a vial (*h*/*w*, 5.4 cm, 2.3 cm) for 1 hr. During this interval, there was considerable physical contact among males, presumably allowing for the transfer of scent from the GRO-exposed males to the non-exposed males. The vial was chilled, and the males that were not exposed directly to GRO (those with intact wings) were used in the trials. Males in the other treatment, i.e., those not exposed to GRO-exposed males, were subject to the same confinement protocol, but in this case the 'other' (non-test) males placed in the vial had not been exposed to GRO.

In experiment 10, we assessed whether GRO exposure per se (i.e., independent of the object exposed) resulted in higher female attraction by comparing female attraction between GRO-exposed *vs* non-exposed males of *B. dorsalis*. Protocol followed that described for experiment 6.

In experiment 11, we monitored female response to GRO-exposed *vs* non-exposed males in a more complex environment, i.e., in the canopy of live, potted plants of *Ficus benjamina* L. Observations were made in a glass aquarium (*l*/*w*/*d*, 50×27×30 cm) that contained two plants; the aquarium was oriented 90° from normal (i.e., with a short side resting on the table top) with the open side covered with a screen mesh. For a given trial, we suspended two screen cages (cylinders, *h*/*w*, 5.0 cm, 3.0 cm) in the canopy that contained four GRO-exposed (after the exposure protocol used in experiment 6) or four non-exposed males (males from both groups were freshly killed through chilling), introduced 40 females, and then recorded the number of females resting directly on, or within 3 cm of, each cage at 1-min intervals over 30 min. The cages were placed at the same locations over all replicates, but the type of male (GRO-exposed or non-exposed) placed at a given location was alternated between successive trials. The aquarium, plants, and cages were rinsed with water after each trial.

Mating Trials: Female and Male Antennal Removal Two sets of mating trials were performed. In the first, we manipulated female ability to perceive olfactory stimuli emitted by males, and in the second, we manipulated male ability to perceive the odor of GRO.

In the first set, we presented GRO-exposed and non-exposed males to intact females or females from which either one or both antennae were removed. Although olfactory sensilla may also occur on the mouthparts (as in *Drosophila*, Shanbhag et al. 1999), the antennae are believed to be the primary olfactory structures in *C. capitata* females (Levinson et al. 1987), and removal of both antennae completely eliminates female response to male pheromone (Nakagawa et al. 1973). For surgery, flies were chilled at 4°C, and the entire antenna(e) was cut off with a finger nail clipper under a dissecting microscope. Intact females were chilled only. Treated males were exposed to GRO (following the method described above) 1 d before testing; both GRO-exposed and non-exposed males were chilled and marked 1 d before testing by placing a small dot of enamel paint on the thorax (the chilling procedure had no apparent adverse effects on subsequent male behavior).

In the second set, we presented intact females with non-exposed and GRO-exposed males from which either one or both antennae were removed, i.e., the two male groups differed in the presence or absence of GRO exposure only and were identical with respect to possible effects of 'surgical trauma' associated with antennal removal. In trials conducted in large screen cages (75×90×120 cm), males lacking both antennae were rarely captured in GRO-baited sticky traps (Shelly, unpublished data), and we presume that removal of both antennae effectively prevented olfactory perception in males. Surgery was performed on males 2 d before testing, and GRO exposure and marking were completed 1 d before testing. All handling procedures were identical to those described above.

Mating Trials: Test Procedure Mating trials were conducted in the laboratory by using screen cages (30 cm cubes) under the same ambient conditions noted above. By using an aspirator, we placed 25 males from each treatment category, GRO-exposed or non-exposed, and 25 females into a cage (females were introduced 15 min after the males). Trials commenced between 0730–0800 hr, and mating pairs were collected at 0.5 hr intervals over a 4-hr period. We established three cages on each of 8 days for trials that involved manipulation of female antennal number (corresponding to intact females and females missing one or two antennae) and two cages on each of 10 d for trials that involved manipulation of male antennal number (corresponding to males missing one or two antennae). Because removal of both female antennae inhibited mating dramatically (a result also noted by Nakagawa et al. 1973 and Levinson et al. 1987), we conducted an additional 17 replicates with these females to gain an adequate sample of mated pairs.

Statistical Analyses In analyzing female attraction, two-way analysis of variance (ANOVA) was used to compare samples in experiments that included covered and uncovered stimulus flies (experiments 1, 2, 6, and 7), and a one-way ANOVA (Student's *t* test for two sample comparisons) was used in experiments using uncovered stimulus flies exclusively (experiments 4, 5, 9, 10, and 11) or paper discs (experiments 3 and 8). In all cases, the Tukey test to identify significant pair-wise differences. In analyzing mating frequency, samples were compared with the Student's *t* test. The data met parametric assumptions in most cases; when they did not, a log transformation was performed to normalize the data. Analyses were performed using SigmaStat Statistical Software (version 2.0). Means + 1 SE are given.

Results

Female Response to Dead Flies: Species- and Sex-Specific Attraction In experiment 1, uncovered or covered males of *C. capitata* and *B. dorsalis* were offered to *C. capitata*

females, and both factors—species identity and presentation method (covered or uncovered)—had effects on female visitation (species, $F_{1,44}$=90.0, P<0.001; presentation, $F_{1,44}$=11.1, P<0.01; Fig. 1a). In addition, the interaction term was significant ($F_{1,44}$=7.0, P<0.05). For both uncovered and covered stimulus flies, females were sighted significantly more often near the *C. capitata* males than near the *B. dorsalis* males. Fewer female sightings were made for covered than uncovered medfly males, whereas no effect of the cloth cover was observed for the oriental fruit fly males.

In experiment 2, uncovered or covered conspecific males and females were offered to *C. capitata* females, and both factors—sex and presentation method—had effects on female visitation (sex, $F_{1,\ 44}$=29.8, P<0.001; presentation, $F_{1,44}$=35.8, P<0.001; Fig. 1b). The interaction term was significant as well ($F_{1,\ 44}$=8.4, P<0.01). For both uncovered and covered stimulus flies, there were more female sightings made near the conspecific males than near conspecific females. For both sexes of stimulus flies, there were fewer female sightings near the covered than the uncovered individuals.

Conspecific males appeared to possess hexane-extractable compounds attractive to females. In experiment 3, there were more female sightings made near paper discs soaked in hexane-wash from male medflies (21.3+3.1) than near discs soaked in hexane only

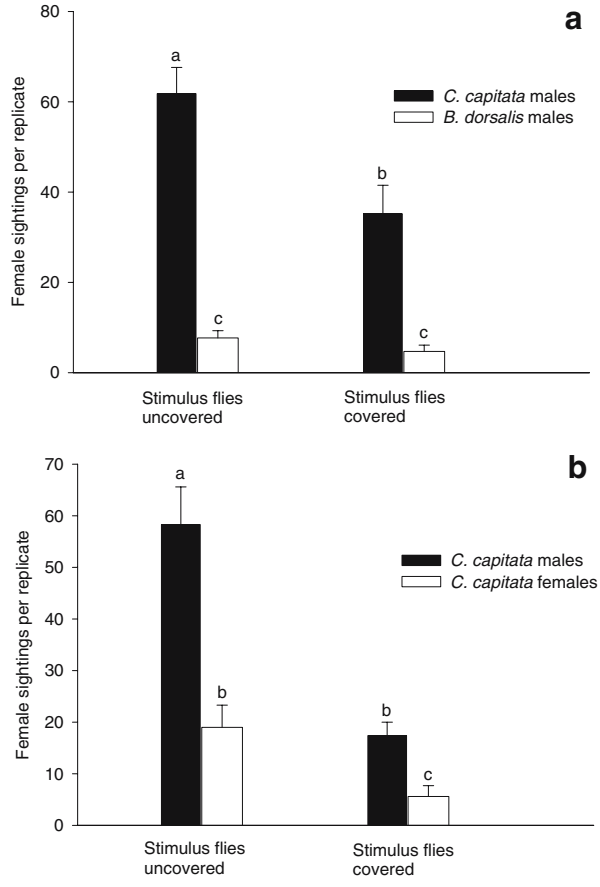

Fig. 1 Sightings of *Ceratitis capitata* females in choice tests near freshly killed **a** males of *C. capitata* and *B. dorsalis* (experiment 1) and **b** males and females of *C. capitata* (experiment 2). *Bar heights* represent mean number (+1 SE) of sightings per replicate (N=12). Means *labeled with same letter* are not significantly different (Tukey test, P=0.05). Note that whereas ANOVA was performed using all sample means, covered and uncovered treatments were presented in separate trials

(10.3+1.8, t=2.4, df=22; P<0.05). In addition, in experiment 4, the number of female sightings recorded for non-hexane-washed males was greater than that recorded for hexane-washed males (85.3+7.5 *vs* 42.1+8.5, respectively, t=2.7, df=22, P<0.05; only trials that used uncovered males were conducted). This difference did not appear to result from female avoidance of the hexane treatment itself. The presence of (covered) hexane-washed males close to non-washed males did not reduce female sightings below that observed for non-washed males presented alone. Female sightings were, in fact, slightly higher for the former group than the latter group, although this difference was not statistically significant (52.8+5.6 *vs* 47.2+4.9 sightings/replicate, t=0.51, df=22, P>0.05, experiment 5).

Female Response to Dead Flies: GRO Exposure to Conspecific Males In experiment 6, uncovered or covered conspecific males that were GRO-exposed or non-exposed were offered to *C. capitata* females, and both factors—GRO treatment and presentation method—had effects on female visitation (GRO exposure, $F_{1,44}$=13.2, P<0.001; presentation, $F_{1,44}$=69.4, P<0.001; Fig. 2a). The interaction term was significant as well ($F_{1,44}$=5.4, P<0.05). For both uncovered and covered stimulus flies, there were more female sightings made near the GRO-exposed males than near the non-exposed males. For both the GRO-exposed and non-exposed stimulus flies, there were fewer female sightings near the covered than the uncovered individuals.

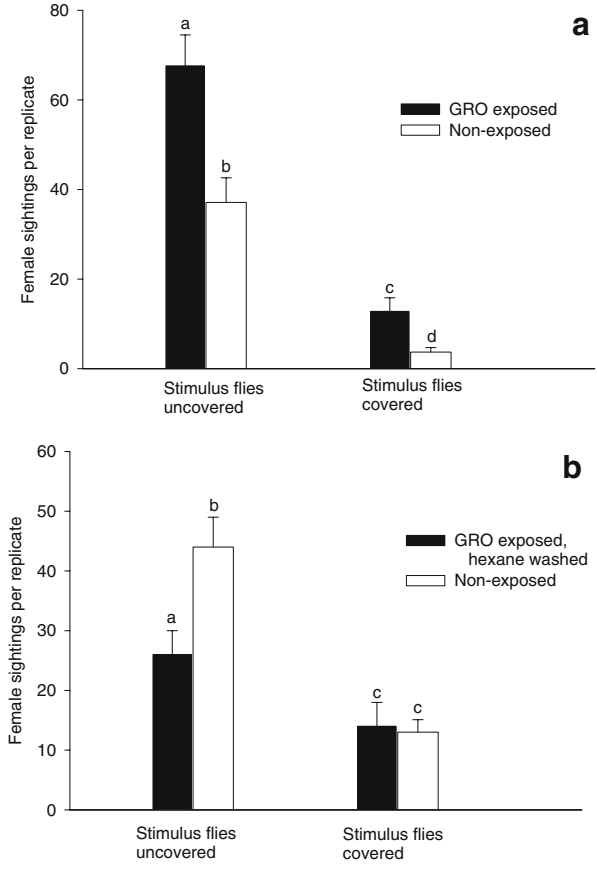

Fig. 2 Sightings of *Ceratitis capitata* females in choice tests near freshly killed males of *C. capitata* that were **a** exposed to GRO or non-exposed (experiment 6) and **b** exposed to GRO and then washed in hexane or non-exposed (experiment 7). *Bar heights* represent mean number of sightings per replicate (N=12); *error bars* represent +1 SE. Means *labeled with same letter* are not significantly different (Tukey test, P=0.05). Note that, whereas ANOVA was performed using all sample means, covered and uncovered treatments were presented in separate trials

Extraction with hexane reduced the attractiveness of GRO-exposed males. In experiment 7, uncovered and covered conspecific males that were GRO-exposed and then hexane-washed or non-exposed were offered to *C. capitata* females, and both factors had effects on female attraction (GRO/hexane treatment, $F_{1,44}=14.4$, $P<0.001$; presentation, $F_{1,\ 44}=6.1$, $P<0.05$; Fig. 2b). The interaction term was significant as well ($F_{1,44}=7.2$, $P<0.05$). For uncovered stimulus flies, there were more female sightings near the non-exposed males than near the hexane-washed, GRO-exposed males. For covered stimulus flies, there was no difference in the number of female sightings for the two male types.

Additional Tests Regarding the Effect of GRO Exposure on Female Response Table 1 presents the results from experiments 8–11. When presented with paper discs that contained extract from hexane-washed males, females were sighted more frequently near discs treated with extracts of GRO-exposed males than non-exposed males (experiment 8). There was also evidence that GRO-induced scent was transferable between males: Females were sighted more frequently near non-exposed males that had been confined with GRO-exposed males than non-exposed males that had been confined with other non-exposed males (experiment 9). Although these tests, along with the previous ones, indicate that GRO has an effect on female response, it does not appear that *C. capitata* females are attracted indiscriminately to GRO-exposed objects independent of their identity. When presented with males of *B. dorsalis*, medfly females were sighted equally near GRO-exposed or non-exposed individuals (experiment 10). When monitored in the larger and more physically complex environment established in the aquaria (experiment 11), females were sighted more frequently near GRO-exposed males than non-exposed males, although this difference was only marginally significant ($P=0.09$).

Mating Trials The first set of mating trials involved manipulation of female antennal number. Both intact females and those lacking one antenna mated preferentially with GRO-exposed males (Table 2). As noted above, females that lacked both antennae showed low mating propensity, and only three matings (one GRO-exposed and two non-exposed males) were recorded for such females over eight cages. Consequently, we set up additional 17 cages with these females, and over all 25 cages, we observed 8 matings with GRO-exposed males and 11 matings with non-exposed males, indicating random choice of mates ($P=0.64$, binomial test).

Table 1 Response of *Ceratitis capitata* females to GRO under varying modes of presentation

Experiment	Mode GRO presentation	GRO treatment	Female sightings per replicate
8	Disc with hexane from medfly males	Exposed	21.2 (4.1)
		Non-exposed	10.3 (1.8)*
9	Medfly males from crowded vials	Exposed	97.3 (7.7)
		Non-exposed	71.4 (4.5)*
10	Oriental fruit fly males	Exposed	6.7 (1.9)
		Non-exposed	7.2 (2.0)**
11	Medfly males in aquarium	Exposed	11.1 (2.5)
		Non-exposed	5.2 (1.4)**

Values represent means (+1 SE) of 12 replicates for experiments 8 and 10 and of 18 and 16 replicates for experiments 9 and 11, respectively. See text for methods used in the individual experiments. Significance level is given for between-treatment comparisons within each experiment, where *$P< 0.05$, **$P>0.05$.

Table 2 Mating trials for *Ceratitis capitata*

	Male treatment		Significance
	GRO-exposed	Non-exposed	
Set 1—Female antennal manipulation (female treatment antennae removed)			
0	12.6 (0.9)	8.6 (0.7)	$P<0.01$
1	12.3 (1.0)	7.8 (0.7)	$P<0.001$
2	8	11	$P>0.05$
Set 2—Male antennal manipulation (male treatment antennae removed)			
1	14.6 (1.1)	8.8 (1.3)	$P<0.01$
2	12.4 (1.0)	8.3 (0.9)	P<0.01

Mean number (SE) of matings per replicate (N=8 for set 1 and N=10 for set 2) are presented with significance level based on the t test, with the exception of the experiment involving removal of both antennae from females where the total numbers of matings are presented over 25 cages, and significance was assessed using the binomial test. In all trials, 25 females and 25 males of each GRO exposure category were placed in screen cages.

In the second set, we manipulated antennal number in males. In these trials, GRO-exposed males achieved significantly more matings than non-exposed males independently of the number of antennae removed from the test males. Among GRO-exposed males, there was no difference in the number of matings obtained by individuals lacking one *vs* two antennae ($P<0.05$). Furthermore, GRO-exposed males accounted for a similar proportion of the total matings in their respective trials (-1 or -2 antennae: 62.8 and 59.9%, respectively, $P>0.05$, test performed using arc sine transformed values).

Discussion

Species- and Sex-Recognition Within the Diptera, profiles of cuticular hydrocarbons, which are primarily contact pheromones with low volatility (Amrein 2004; Vogt 2005), differ among closely related species (e.g., *Drosophila*, Etges and Jackson 2001; *Tabanus*, Hoppe et al. 1990; and *Anopheles*, Carlson et al. 1997) and have an important role in species recognition and reproductive isolation. In contrast, the present study indicates that females of *C. capitata* distinguished conspecific from heterospecific males based on chemical cues associated with volatile compounds. Females displayed species-specific discrimination even when the test males were covered and could not be seen or touched directly. Female medflies were only weakly attracted to *B. dorsalis* males, and their response was independent of the manner in which the heterospecific males were presented (i.e., covered or uncovered). In contrast, females showed a significantly higher attraction to uncovered than covered conspecific males, indicating that visual and/or contact cues influenced female response. Species-specific differences in cuticular hydrocarbon profiles have been reported for larvae of several tephritid species (Carlson and Yocom 1986; Sutton and Steck 1994), and, along with the volatile attractants suggested by the present findings, contact pheromones may also function in species recognition in the Tephritidae.

Our tests also revealed that female medflies use volatile olfactory cues to distinguish sex among conspecific individuals. As with species recognition, *C. capitata* females distinguished conspecific individuals by sex even when these individuals were covered and could not be seen or touched. For both sexes, female attraction was greater for

uncovered individuals, indicating that visual and/or contact chemical cues also influenced female response. Sexual differences in cuticular hydrocarbons have been reported for other Diptera (e.g., *Drosophila*, Suvanto et al. 2000; *Fannia*, Uebel et al. 1977) but, to our knowledge, have not been documented for any tephritid species.

Discrimination Among Conspecific Males Trials that tested female response to freshly killed flies or paper discs indicated that females distinguished GRO-exposed and non-exposed males on the basis of olfactory cues. Even when the males were covered, females were sighted more often near GRO-exposed than non-exposed males, and females preferred to settle near paper discs treated with extract from GRO-exposed than non-exposed males. Washing GRO-exposed males in hexane eliminated female preference for these males, whereas confining non-exposed males with GRO-exposed males increased the attractiveness of non-exposed males, suggesting transfer of substances from the GRO-exposed flies.

Surgical removal of the antennae of females allowed a direct test of the role of olfaction on mate choice. Unfortunately, as noted previously (Nakagawa et al. 1973; Levinson et al. 1987), females lacking both antennae do not mate readily, and we obtained a total of only 19 matings that involved such females. These matings showed no deviation from random choice, with GRO-exposed and non-exposed males, accounting for 42 and 58% of the matings, respectively. Costanzo and Monteiro (2007) reported a similar finding in a nymphalid butterfly, where females with 'blocked' antennae (covered with nail varnish) mated indiscriminately with control males emitting normal odors and treated males whose scent producing glands were blocked and rendered non-functional (see also Pivnick et al. 1992). Based on our results, it appears that the previously noted GRO-mediated increase in male mating success (Shelly 2001; Shelly et al. 2004b) depends on female perception of male-derived olfactory cues. The converse approach—antennal removal from males—showed that male ability to perceive the odor of GRO was not necessary for enhanced mating performance. Males exposed to GRO after removal of both antennae achieved significantly more matings than non-exposed (but antenna-less) males. It thus appears that GRO treatment confers a mating advantage through an external phenomenon, i.e., an alteration in body scent, rather than internal processing, e.g., utilization in pheromone synthesis after absorption of airborne molecules.

Two studies on female attraction to pheromone-calling males further support this interpretation. As noted previously, Shelly (2001), working in a field cage, established leks comprised of GRO-exposed or non-exposed males, respectively, and monitored male calling and female visitation. These observations showed that female visitation was directly related to the calling activity associated with given leks (independent of male type) and, as such, was apparently influenced by signaling level per se independent of signal composition (quality). Additionally, Papadopoulos et al. (2006), working in a laboratory wind tunnel, reported that similar numbers of females arrived to spheres emitting the pheromone odor of GRO-exposed or non-exposed males, respectively. The absence of female preference in this experiment suggests that the increased mating success of GRO-exposed males does not reflect the production of a more attractive, long-range pheromonal signal by GRO-treated males. Instead, it appears that GRO's impact is greatest during close-range courtship.

Briceño et al. (2007) found that GRO-exposed and non-exposed males displayed the same behavioral elements of courtship and that wild (but not mass-reared) females accepted GRO-exposed males, not only more frequently but also more rapidly than non-exposed males. The absence of major behavioral differences between males in the two treatments suggests that other sensory modalities, i.e., auditory or olfactory, may be responsible for the

GRO-mediated increase in courtship success. During courtship, males produce sounds via rapid vibration and rhythmic forward-and-backward movements of the wings (Sivinski et al. 1989), but the significance of such acoustic signals has not been investigated experimentally. Whereas auditory cues cannot be dismissed, the present study supports the hypothesis that females may discriminate among courting males, in part at least, on the basis of GRO-related body scent.

Implications for Understanding Female Mate Selection in Natural Populations

The mating success of male medflies is enhanced through exposure, not only to GRO (Shelly 2001) but also to certain fruits (oranges and guavas; Papadopoulos et al. 2001; Shelly et al. 2004a; Shelly and Villalobos 2004), tree bark (guava, Shelly and Villalobos 2004), and other commercially available oils (e.g., orange oil, Shelly et al. 2004a; manuka oil, Shelly et al. 2007b). Although the active compound is not known with certainty, all these substances (GRO, Takeoka et al. 1990; oranges, MacLeod et al. 1988; Nishida et al. 2000; orange oil, Takeoka et al. 1990; guava bark and fruits, MacLeod and De Troconis 1982; Shelly and Villalobos 2004; manuka oil, Douglas et al. 2004) contain the hydrocarbon sesquiterpene α-copaene, which is known to be a powerful attractant to male medflies (Warthen and McInnis 1989; Flath et al. 1994a,b). Importantly, exposure to α-copaene alone was found to increase the mating success of *C. capitata* males (Shelly 2001). As α-copaene is widely distributed among plants, it appears reasonable to assume that this compound (and possibly other related compounds) influences the mating success of *C. capitata* males in the wild.

One pattern that has emerged from studies of α-copaene-containing substances is that, for the commercially available oils, exposure to the aroma alone is sufficient to boost male performance in *C. capitata*, whereas, for the fruits and bark, physical contact is required for mating enhancement. For example, males exposed to the odor of orange oil enjoy increased mating success, whereas males provided oranges in screened containers (thus preventing contact) gain no mating advantage (Shelly et al. 2004a). This trend may reflect the higher concentration of α-copaene (and related compounds) in the oils than in the plants. The concentration of α-copaene in orange oil is 0.2% (S. Young, personal communication) compared to only 0.7 μg/g for the peel of the fruit (Nishida et al. 2000). Based on these observations, it appears likely that, in the wild, male medflies must touch α-copaene-containing plant structures to gain a mating advantage. We are planning future studies to address whether contact of these structures alone (i.e., without feeding) enhances male mating success.

Adaptive Benefit of Female Preference for Scented Males The adaptive benefit of female preference for scented males remains unknown. In a laboratory study (Shelly 2005), females mated with GRO-exposed males did not lay significantly more eggs or live significantly longer than females mated with non-exposed males. Likewise, whether or not a male was exposed to GRO before mating had no significant effect on the proportion of eggs that hatched or the proportion of eggs that yielded pupae (Shelly 2005). Thus, it does not appear that GRO-exposed males transfer some material resource that directly increases female fecundity or vigor. Alternatively, the emission of particular phytochemically derived scents by males may indicate a superior ability to locate the appropriate plants in the habitat; that is, by selecting males with, presumably, an α-copaene-derived scent over others, female medflies may increase the odds that their sons will have high ability to locate sources of α-copaene and hence enjoy high mating success. This scenario constitutes a case

of runaway selection, whereby female choice provides indirect benefits via a trait that confers an advantage to her sons in sexual competition but is arbitrary with respect to offspring viability (Andersson 1994).

If this runaway selection hypothesis is valid, the honesty inherent in a scent-based signal would appear to vary inversely with the ease with which sources of α-copaene were locatable. In cases where such sources are relatively common, a larger proportion of males might find them by chance alone, and female selection of scented males may be less likely to yield sons with high searching capacity. This situation raises the interesting, more general, possibility that female choice based on exogenously derived, olfactory signals (body scent) may be less reliable than choice based on endogenously produced pheromonal signals, e.g., where mate selection is based on the chemical composition of male pheromone, which may serve as an honest indicator of, for example, developmental stability (Thornhill 1991), immunocompetence (Rantala et al. 2002), or dominance status (Moore 1988).

Acknowledgments We thank Eric Jang, Jocelyn Millar, David Robacker, and Keng-Hong Tan for technical advice. Sylvia Young of Citrus and Allied Essences Ltd. supplied information regarding the chemical composition of ginger root oil. Eric Jang, Jocelyn Millar, and Boaz Yuval provided comments on an earlier draft.

References

Amrein, H. 2004. Pheromone perception and behavior in *Drosophila*. *Curr. Opinion Neurobiol* 14:435–442.
Andersson, M. 1994. Sexual Selection. Princeton University Press, Princeton, USA.
Arita, L. H., and Kaneshiro, K. Y. 1986. Structure and function of the rectal epithelium and anal glands during mating behavior in the Mediterranean fruit fly male. *Proc. Hawaiian Entomol. Soc* 26:27–30.
Bartelt, R. J., Schaner, A. M., and Jackson, L. L. 1988. Aggregation pheromones in *Drosophila borealis* and *Drosophila littoralis*. *J. Chem. Ecol* 14:1319–1327.
Bernays, E. A., and Chapman, R. F. 1994. Host-Plant Selection by Phytophagous Insects. Chapman and Hall, New York.
Briceño, D., Eberhard, W. G., and Shelly, T. E. 2007. Male courtship behavior in *Ceratitis capitata* (Diptera: Tephritidae) that have received aromatherapy with ginger root oil. *Fla. Entomol* 90:175–179.
Carlson, D. A., Reinert, J. F., Bernier, U. R., Sutton, B. D., and Seawright, J. A. 1997. Analysis of the cuticular hydrocarbons among species of the *Anopheles quadrimaculatus* complex (Diptera: Culicidae). *J. Am. Mosq. Control Assoc* Suppl.:103–111.
Carlson, D. A., and Yocom, S. R. 1986. Cuticular hydrocarbons from six species of fruit flies. *Arch. Insect Biochem. Physiol* 3:397–412.
Connor, W. F., Eisner, T., Vandermeer, R. K., Guerrero, A., and Meinwald, J. 1981. Precopulatory sexual interaction in an arctiid moth (*Utetheisa ornatrix*): the role of a pheromone derived from dietary alkaloids. *Behav. Ecol. Sociobiol* 9:227–235.
Coracini, M., Bengtsson, M., Liblikas, I., and Witzgall, P. 2004. Attraction of codling moth males to apple volatiles. *Entomol. Exp. Appl* 110:1–10.
Costanzo, K., and Monteiro, A. 2007. The use of chemical and visual cues in female choice in the butterfly *Bicyclus anynana*. *Proc. Roy. Soc. Lond. B* 274:845–851.
Coyne, J. A., Crittenden, A. P., and Mah, K. 1994. Genetics of a pheromonal difference contributing to reproductive isolation in *Drosophila*. *Science* 265:1461–1464.
Douglas, M. H., Van Klink, J. W., Smallfield, B. M., Perry, N. B., Anderson, R. E., Johnstone, P., and Weavers, R. T. 2004. Essential oils from New Zealand manuka: triketone and other chemotypes of *Leptospermum scoparium*. *Phytochemistry* 65:1255–1264.
Dowd, P. F., and Bartelt, R. J. 1991. Host-derived volatiles as attractants and pheromone synergists for dried fruit beetle, *Carpophilus hemipterus*. *J. Chem. Ecol* 17:285–308.
Etges, W. J., and Jackson, L. L. 2001. Epicuticular hydrocarbon variation in *Drososphila mojavensis* cluster species. *J. Chem. Ecol* 27:2125–2149.

FÉRON, M. 1962. L'instinct de reproduction chez la mouche Mediterranéan des fruits *Ceratitis capitata* Wied. (Diptera: Trypetidae). Comportement sexuel. Comportement de ponte. *Rev. Pathol. Veg. Entomol. Agric. Fr* 41:1–129.

FLATH, R. A., CUNNINGHAM, R. T., MON, T. R., and JOHN, J. O. 1994a. Additional male Mediterranean fruit fly (*Ceratitis capitata* Wied.) attractants from angelica seed oil (*Angelica archangelica* L.). *J. Chem. Ecol* 20:1969–1984.

FLATH, R. A., CUNNINGHAM, R. T., MON, T. R., and JOHN, J. O. 1994b. Male lures for Mediterranean fruit fly (*Ceratitis capitata* Wied.): structural analogues of α-copaene. *J. Chem. Ecol* 20:2595–2609.

GINZEL, M. D., and HANKS, L. M. 2005. Role of host plant volatiles in mate location for three species of longhorned beetles. *J. Chem. Ecol* 31:213–217.

GREENFIELD, M. D., SHELLY, T. E., and DOWNUM, K. R. 1987. Variation in host plant quality: implications for territoriality in a desert grasshopper. *Ecology* 68:828–838.

HANKS, L. M., MILLAR, J. G., and PAINE, T. D. 1996. Mating behavior of the eucalyptus longhorned borer (Coleoptera: Cerambycidae) and the adaptive significance of long "horns". *J. Insect Behav* 9:383–393.

HEATH, R. R., LANDOLT, P. J., TUMLINSON, J. H., CHAMBERS, D. L., MURPHY, R. E., DOOLITTLE, D. E., DUEBEN, D. B., SIVINSKI, J., and CALKINS, C. O. 1991. Analysis, synthesis, formulation and field testing of the three major components of male Mediterranean fruit fly pheromone. *J. Chem. Ecol* 17:1925–1940.

HENDRICHS, J., and HENDRICHS, M. A. 1990. Mediterranean fruit fly (Diptera: Tephritidae) in nature: location and diel pattern of feeding and other activities on fruiting and nonfruiting hosts and nonhosts. *Ann. Entomol. Soc. Am* 83:632–641.

HOPPE, K. L., DILLWITH, J. W., WRIGHT, R. E., and SZUMLAS, D. E. 1990. Identification of horse flies (Diptera: Tabanidae) by analysis of cuticular hydrocarbons. *J. Med. Entomol* 27:480–486.

HOVORKA, O., KINDL, J., KALINOVA, B., KNÍŽEK, M., VRKOČOVÁ, and KOUTEK, B. 2005. The role of beetle and host volatiles in host colonization in the European oak bark beetle, *Scolytus intricatus* (Ratzeburg) (Col., Scolytidae). *J. Appl. Entomol* 129:221–226.

HUGHES, P. R., and RENWICK, J. A. A. 1977. Hormonal and host factors stimulating pheromone synthesis in female western pine beetles *Dendroctonus brevicomis*. *Physiol. Entomol* 2:289–292.

JAFFE, K., SANCHEZ, P., CERDA, H., HERNANDEZ, J. A., JAFFE, R., URDANETA, N., GUERRA, G., MARTINEZ, R., and MIRAS, B. 1993. Chemical ecology of the palm weevil *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae): attraction to host plants and to a male produced pheromone. *J. Chem. Ecol* 19:1703–1720.

JANG, E. B., LIGHT, D. M., BINDER, R. G., FLATH, R. A., and CARVALHO, L. A. 1994. Attraction of female Mediterranean fruit flies to the five major components of male-produced pheromone in a laboratory flight tunnel. *J. Chem. Ecol* 20:9–20.

JANG, E. B., LIGHT, D. M., FLATH, R. A., NAGATA, J. T., and MON, T. R. 1989. Electroantennogram responses of the Mediterranean fruit fly, *Ceratitis capitata*, to identified volatile constituents from calling males. *Entomol. Exp. Appl* 50:7–19.

KATSOYANNOS, B. I., KOULOUSSIS, N. A., and PAPADOPOULOS 1997. Response of *Ceratitis capitata* to citrus chemicals under semi-natural conditions. *Entomol. Exp. Appl* 82:181–188.

KRASNOFF, S. B., and DUSSOURD, D. E. 1989. Dihydropyrrolizidine attractants for arctiid moths that visit plants containing pyrrolizidine alkaloids. *J. Chem. Ecol* 15:47–60.

LANDOLT, P. J., and PHILLIPS, T. W. 1997. Host plant influences on sex pheromone behavior of phytophagous insects. *Annu. Rev. Entomol* 42:371–391.

LANDOLT, P. J., HEATH, R. R., MILLAR, J. G., DAVIS-HERNANDEZ, K. M., DUEBEN, D. B., and WARD, K. E. 1994. Effects of host plant, *Gossypium hirsutum* L., on sexual attraction of cabbage looper moths, *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae). *J. Chem. Ecol* 20:2959–2974.

LEVINSON, H. Z., LEVINSON, A. R., and SCHÄFER, K. 1987. Pheromone biology of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) with emphasis on the functional anatomy of the pheromone glands and antennae as well as mating behaviour. *J. Appl. Entomol* 104:448–461.

LOFSTEDT, C., VICKERS, N. J., ROELOFS, W. L., and BAKER, T. C. 1989. Diet related courtship success in the Oriental fruit moth, *Grapholita molesta* (Tortricidae). *Oikos* 55:402–408.

MACLEOD, A. J., and DE TROCONIS, N. G. 1982. Volatile flavour components of guava. *Phytochemistry* 21:1339–1342.

MACLEOD, A. J., MACLEOD, G., and SUBRAMANIAN, G. 1988. Volatile aroma constituents of orange. *Phytochemistry* 27:2185–2188.

MARCILLAC, F., and FERVEUR, J.-F. 2004. A set of female pheromones affects reproduction before, during and after mating in *Drosophila*. *J. Exp. Biol* 207:3927–3933.

MOORE, A. J. 1988. Female preferences, male social status, and sexual selection in *Nauphoeta cinerea*. *Anim. Behav* 36:303–305.

NAKAGAWA, S., FARIAS, G. J., SUDA, D., and CHAMBERS, D. L. 1973. Mating behavior of the Mediterranean fruit fly following excision of the antennae. *J. Econ. Entomol* 66:583–584.

NISHIDA, R., SHELLY, T. E., WHITTIER, T. S., and KANESHIRO, K. Y. 2000. Alpha- copaene, a potential rendezous cue for the Mediterranean fruit fly, *Ceratitis capitata?*. *J. Chem. Ecol* 26:87–100.

PAPADOPOULOS, N. T., KATSOYANNOS, B. I., KOULOUSSIS, N. A., and HENDRICHS, J. 2001. Effect of orange peel substances on mating competitiveness of male *Ceratitis capitata*. *Entomol. Exp. Appl* 99:253–261.

PAPADOPOULOS, N. T., SHELLY, T. E., NIYAZI, N., and JANG, E. B. 2006. Olfactory and behavioral mechanisms underlying enhanced mating competitiveness following exposure to ginger root oil and orange oil in males of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *J. Insect Behav* 19:403–418.

PIVNICK, K. A., LAVOIE-DORNIK, J., and MCNEIL, J. N. 1992. The role of the androconia in the mating behaviour of the European skipper, *Thymelicus lineola*, and evidence for a male sex pheromone. *Physiol. Entomol* 17:260–268.

PLISKE, T. E. 1975. Courtship behavior and use of chemical communication by males of certain species of ithomiinae butterflies (Nymphalidae: Lepidoptera). *Ann. Entomol. Soc. Am* 68:935–942.

PROKOPY, R. J., and HENDRICHS, J. 1979. Mating behavior of *Ceratitis capitata* in field cages on host trees. *Ann. Entomol. Soc. Am* 72:642–648.

QUIROZ, A., ORTEGA, F., RAMÍREZ, C. C., WADHAMS, L. J., and PINILLA, K. 2005. Response of the beetle *Hylastinus obscurus* Marsham (Coleoptera: Scolytidae) to red clover (*Trifolium pretense* L.) volatiles in a laboratory olfactometer. *Environ. Entomol* 34:690–595.

RANTALA, M. J., JOKINEN, I., KORTET, R., VAINIKKA, A., and SUHONEN 2002. Do pheromones reveal male immunocompetence? *Proc. Roy. Soc. Lond. B* 269:1681–1685.

SHANBHAG, S., MULLER, B., and STEINBRECHT, A. 1999. Atlas of olfactory organs of *Drosophila melanogaster*. 1. Types, external organization, innervation and distribution of olfactory sensilla. *Int. J. Insect Morphol. Embryol* 28:377–397.

SHELLY, T. E. 2001. Exposure to α-copaene and α-copaene-containing oils enhances the mating success of male Mediterranean fruit flies (Diptera: Tephritidae). *Ann. Entomol. Soc. Am* 94:497–502.

SHELLY, T. E. 2005. Does mating with ginger root oil-exposed males confer fitness benefits to female Mediterranean fruit flies, *Ceratitis capitata* (Diptera: Tephritidae)? *Proc. Hawaiian Entomol. Soc* 37: 65–71.

SHELLY, T. E., and VILLALOBOS, E. 2004. Host plant influence on the mating success of male Mediterranean fruit flies: variable effects within and between individual plants. *Anim. Behav* 68:417–426.

SHELLY, T. E., and KENNELLY, S. 2007. Settlement patterns of Mediterranean fruit flies in the tree canopy: an experimental analysis. *J. Insect Behav* 20:453–472.

SHELLY, T. E., EDU, J., SMITH, E., HOFFMAN, K., WAR, M., SANTOS, R., FAVELA, A., GARAGLIANO, R., IBEWIRO, B., and MCINNIS, D. O. 2007a. Aromatherapy on a large scale: exposing entire holding rooms to ginger root oil increases the mating competitiveness of sterile males of the Mediterranean fruit fly. *Entomol. Exp. Appl* 123:193–201.

SHELLY, T. E., COWAN, A. N., EDU, J., and PAHIO, E. 2007b. Mating success of male Mediterranean fruit flies following exposure to two sources of α-copaene, manuka oil and mango. *Fla. Entomol*. (in press) .

SHELLY, T. E., DANG, C., and KENNELLY, S. 2004a. Exposure to orange (*Citrus sinensis* L.) trees, fruit, and oil enhances mating success of male Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]). *J. Insect Behav* 17:303–315.

SHELLY, T. E., MCINNIS, D. O., PAHIO, E., and EDU, J. 2004b. Aromatherapy in the Mediterranean fruit fly (Diptera: Tephritidae): sterile males exposed to ginger root oil in pre-release, storage boxes display increased mating competitiveness in field-cage trials. *J. Econ. Entomol* 97:846–853.

SIVINSKI, J., CALKINS, C. O., and WEBB, J. C. 1989. Comparisons of acoustic courtship signals in wild and laboratory reared Mediterranean fruit fly *Ceratitis capitata*. *Fla. Entomol* 72:212–214.

SUTTON, B. D., and STECK, G. J. 1994. Disrimination of Caribbean and Mediterranean fruit fly larvae (Diptera: Tephritidae) by cuticular hydrocarbon analysis. *Fla. Entomol* 77:231–237.

SUVANTO, L., LIIMATAINEN, J. O., TREGENZA, T., and HOIKKALA, A. 2000. Courtship signals and mate choice of the flies of inbred *Drosophila montana* strains. *J. Evol. Biol* 13:583–592.

TAKEOKA, G., FLATH, R. A., MON, T. R., BUTTERY, R. G., TERANISHI, R., GUNTERT, M., LAUTAMO, R., and SZEJTLI, J. 1990. Further applications of permethylated β-cyclodextrin capillary gas chromatographic columns. *J. High Resol. Chromatogr* 13:202–206.

TANAKA, N., STEINER, L. F., OHINATA, K., and OKAMOTO, R. 1969. Low-cost larval rearing medium for mass-production of oriental and Mediterranean fruit flies. *J. Econ. Entomol* 62:967–968.

THORNHILL, R. 1991. Female preference for the pheromone of males with low fluctuating asymmetry in the Japanese scorpionfly (*Panorpa japonica*: Mecoptera). *Behav. Ecol* 3:277–282.

THORNHILL, R., and ALCOCK, J. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, USA.

UEBEL, E. C., SONNET, P. E., MENZER, R. E., MILLER, R. W., and LUSBY, W. R. 1977. Mating-stimulant pheromone and cuticular lipid constituents of the little house fly, *Fannia canicularis* (L.). *J. Chem. Ecol* 3:269–278.

VOGT, R. G. 2005. Molecular basis of pheromone detection in insects, pp. 753–804, in L. I. Gilbert, K. Iatro, and S. Gill (eds.). Comprehensive Insect Physiology, Biochemistry, Pharmacology, and Molecular Biology. Vol. 3: EndocrinologyElsevier, London.

WARTHEN, J. D., and MCINNIS, D. O. 1989. Isolation and identification of male medfly attractive components in *Litchi chinensis* stems and *Ficus* spp. stem exudates. *J. Chem. Ecol* 15:1931–1946.

WHITTIER, T. S., NAM, F. Y., SHELLY, T. E., and KANESHIRO, K. Y. 1994. Male courtship success and female discrimination in the Mediterranean fruit fly (Diptera: Tephritidae). *J. Insect Behav* 7:159–170.

WHITTIER, T. S., KANESHIRO, K. Y., and PRESCOTT, L. D. 1992. Mating behavior of Mediterranean fruit flies (Diptera: Tephritidae) in a natural environment. *Ann. Entomol. Soc. Am* 85:214–218.